Validation & Assay Performance Summary

GeneBLAzer® VDR DA Cells & Assay Kit

GeneBLAzer® VDR UAS-*bla* HEK 293T Cells

Cat. no. K1417, K1700

Target Description

Vitamin D receptor (VDR) is an important member of the nuclear hormone receptor superfamily; it plays a critical role in calcium homeostasis and cancer.

Cell Line Description

GeneBLAzer® VDR DA(Division Arrested) cells and VDR-UAS-*bla* HEK 293T cells contain the ligand-binding domain (LBD) of the human Vitamin D receptor (VDR) fused to the DNA-binding domain of GAL4 stably integrated in the GeneBLAzer® UAS-*bla* HEK 293T cell line. GeneBLAzer® UAS-*bla* HEK 293T cells stably express a beta-lactamase reporter gene under the transcriptional control of an upstream activator sequence (UAS). When an agonist binds to the LBD of the GAL4 (DBD)-VDR (LBD) fusion protein, the protein binds to the UAS, resulting in expression of beta-lactamase. Division Arrested (DA) cells are available in two configurations- an Assay Kit (which includes cells and sufficient substrate to analyze 1 x 384-well plate), and a tube of cells sufficient to analyze 10 x 384-well plates.

DA cells are irreversibly division arrested using a low-dose treatment of Mitomycin-C, and have no apparent toxicity or change in cellular signal transduction. Both VDR DA cells and VDR-UAS-*bla* HEK 293T cells are functionally validated for Z' and EC_{50} concentrations of Calcitriol (1α, 25-dihydroxyvitamin D3) (Figure 1). In addition, VDR-UAS-*bla* HEK 293T cells have been tested for assay performance under variable conditions, including DMSO concentration, cell number, stimulation time, and substrate loading time.

Validation Summary

Performance of this assay was validated under optimized conditions in 384-well format using LiveBLAzer™-FRET B/G Substrate.

1. **Primary agonist dose response under optimized conditions (n=6)**

	DA	Dividing
Calcitriol EC_{50}	0.57nM	0.45nM
Z'-Factor (EC_{100})	0.84	0.88

Response Ratio	= 10
Optimum cell no.	= 20K cells/well
Optimum [DMSO]	= up to 1%
Stimulation Time	= 5 hours
Max. [Stimulation]	= 50 nM

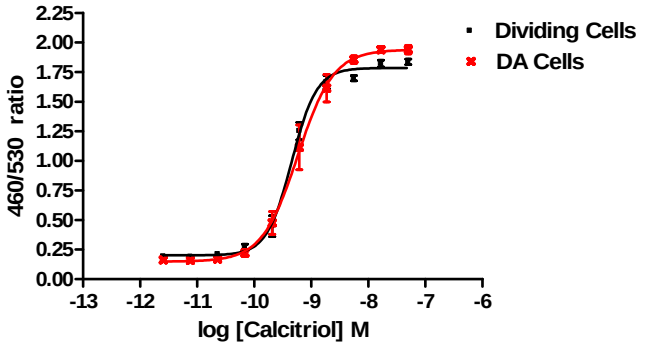
2. **Cell culture and maintenance**
   See Cell Culture and Maintenance Section and Table 1

Assay Testing Summary

3. **Assay performance with variable cell number**
4. **Assay performance with variable DMSO concentration**
5. **Assay performance with variable stimulation time**
6. **Assay performance with variable substrate loading time**

Primary Agonist Dose Response

Figure 1 — VDR DA and VDR-UAS-*bla* HEK 293T dose response to Calcitriol under optimized conditions



VDR DA cells and VDR-UAS-*bla* HEK 293T cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were stimulated with a dilution series of Calcitriol in the presence of 0.5% DMSO for 5 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate (1µM final concentration of CCF4-AM) for 2 hours. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and plotted for each replicate against the concentrations of Calcitriol (n=6 for each data point).

Dividing Cell Culture and Maintenance

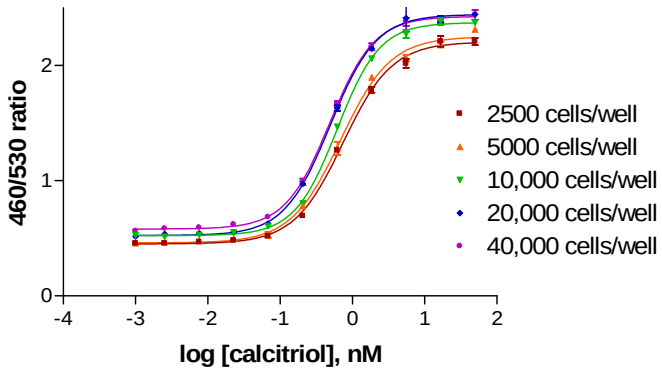
Dividing cells should be maintained at between 5 and 90% confluency in complete growth media and in a humidified incubator at 37ºC and 5% CO_2. Split dividing cells at least twice a week. Do not allow dividing cells to reach confluence.

Table 1 – Dividing Cell Culture and Maintenance

Component	Growth Medium (–)	Growth Medium (+)	Assay Medium	1X Matrigel Matrix	Freeze Medium
DMEM, w/ GlutaMAX™	90%	90%	—	99.75%	—
DMEM, phenol red-free	—	—	98%	—	—
Dialyzed FBS **Do not substitute!**	10%	10%	—	—	—
CD treated FBS	—	—	2%	—	—
NEAA	0.1 mM	0.1 mM	0.1 mM	—	—
Sodium Pyruvate	1mM	1mM	1 mM	—	—
HEPES (pH 7.3)	25 mM	25 mM	—	—	—
Hygromycin antibiotic	—	80 µg/mL	—	—	—
Zeocin® antibiotic	—	80 µg/mL	—	—	—
Penicillin	100 U/mL	100 U/mL	100 U/mL	—	—
Streptomycin	100 µg/mL	100 µg/mL	100 µg/mL	—	—
Matrigel	—	—	—	0.25%	—
Recovery™ Cell Culture Freezing Medium	—	—	—	—	100%

Assay Performance with Variable Cell Number

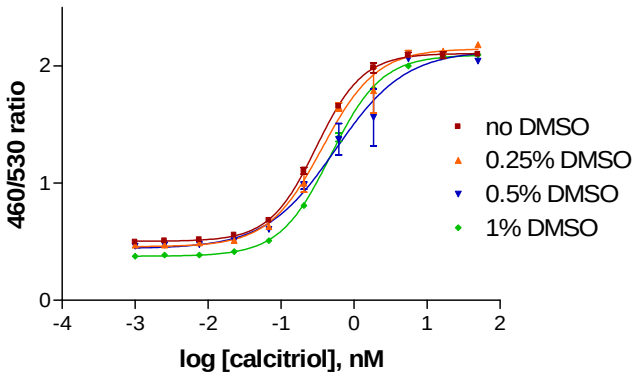
Figure 2 – Calcitriol dose response with 2.5, 5, 10, 20 and 40K cells per well



VDR-UAS-*bla* HEK293T cells were plated at 2500, 5000, 10,000, 20,000, or 40,000 cells/well in a 384-well format the day of the assay in 0.5% DMSO. Cells were stimulated with Calcitriol for 5 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate (1µM final concentration of CCF4-AM) for 1.5 hours. Fluorescence emission values at 460 nm and 530 nm for the various cell numbers were obtained using a standard fluorescence plate reader and the Ratios plotted against the indicated concentrations of Calcitriol (n=8 for each data point).

Assay Performance with variable DMSO concentration

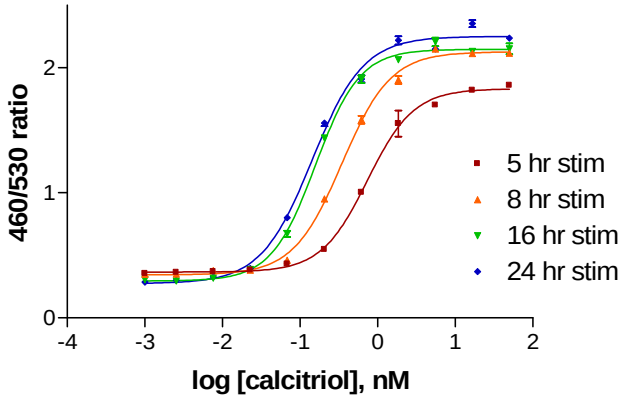
Figure 3 – VDR-UAS-*bla* HEK293T dose response to Calcitriol with 0, 0.25, 0.5 and 1% DMSO.



VDR-UAS-*bla* HEK293T cells (20,000 cells/well) were plated the day of the assay in a 384-well black-walled tissue culture assay plate. Calcitriol was then added to the plate over the indicated concentration range. DMSO was added to the assay at concentrations from 0% to 1%. Cells were stimulated for 5 hrs with agonist and loaded for 1.5 hours with LiveBLAzer™-FRET B/G Substrate (1µM final concentration of CCF4-AM). Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the Ratios are shown plotted for each DMSO concentration against the indicated concentrations of Calcitriol (n=8 for each data point).

Assay Performance with variable stimulation time

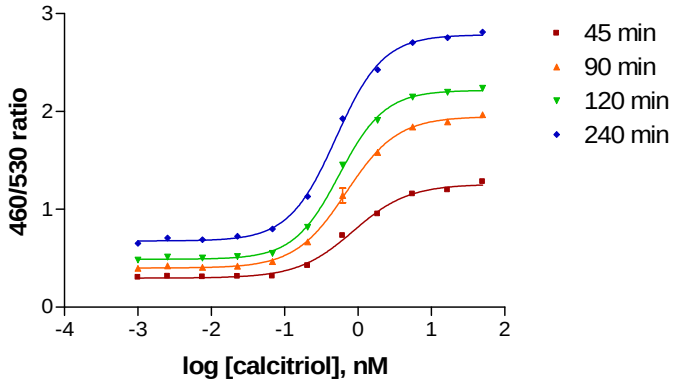
Figure 4 – VDR-UAS-*bla* HEK293T dose response to Calcitriol with 5, 8, 16, and 24 hour stimulation times



VDR-UAS-*bla* HEK293T cells (20,000 cells/well) were plated the day of the assay in a 384-well black-walled tissue culture assay plate in 0.5% DMSO. Calcitriol was then added to the plate over the indicated concentration range for 5, 8, 16, and 24 hours and then loaded for 1.5 hours with LiveBLAzer™-FRET B/G Substrate (1µM final concentration of CCF4-AM). Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the Ratios plotted against the indicated concentrations of Calcitriol (n=8 for each data point).

Assay Performance with variable substrate loading time

Figure 5 – VDR-UAS-*bla* HEK293T dose response to Calcitriol with 45, 90, 120 and 240 minute substrate loading time



VDR-UAS-*bla* HEK293T cells were plated at 20,000 cells/well in a 384-well format the day of the assay in 0.5% DMSO. Cells were stimulated with Calcitriol for 5 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate (1µM final concentration of CCF4-AM) for either 45, 90, 120, or 240 minutes. Fluorescence emission values at 460 nm and 530 nm for the various loading times were obtained using a standard fluorescence plate reader and the Ratios plotted against the indicated concentrations of Calcitriol (n=16 for each data point).